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(57) Abstract

Subject of the invention are non-stoichiometric compounds - with a 1:0.3 to 4.0 mole ratio - of Primycin or its components formed with a cyclodextrin or a cyclodextrin derivative or with mixture thereof.

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PRIMYCIN COMPOUND WITH CYCLODEXTRIN

This invention relates to non-stoichiometric compounds with a 1:0.3 to 4.0 mole ratio of Primycin formed with a cyclodextrin, or a cyclodextrin-derivative, or mixtures thereof of formula I

- where X means 0.3 to 4.0 -

to the process of their preparation and to pharmaceutical compositions containing them.

It is known that the antibiotic agent, Primycin (5-/18-/α-D-arabinofuranosyloxy-/-2-butyl-3,7,11,15,19,21,23,25,27-nonahydroxy-4,16,32,34-tetramethyl-1-oxo-oxacyclohexatriakonta-16,32-diene-35-yl/-4-hydroxyhexyl/guanidinium sulphate/ /Vályi-Nagy T., Uri J., Szilágyi I.:Nature 174 1105 (1954)/ and its components (Canadian Patent Specification No. 1.314.285.) are effective against Gram-positive micro-organisms, including strains which are resistant and polyresistant to other active ingredients, against pathogenic and apathogenic mycobacterium strains, blastomycetes and hyphomycetes, vibrios, certain algae, protozoons, macroviruses, and in higher concentrations even against Gram-negative bacteria /Uri J., Actor P.: Antibiotics 32 , 11 1207 to 1209 (1979)/. It was reported among the valuable pharmaceutical active ingredients /Hansch,C. Editor: Comprehensive Medicinal Chemistry, Pergamon Press 1990 Vol.6./. However, up to date it was to find on the market only in the form of a hydroalcoholic gel, under the name of Ebrimycin gel, containing 0.2 mass % active ingredient, although the therapy would require many other applications in appropriate pharmaceutical formulations.

For the use in ophthalmology, otorhinolaringology, surgery, urology, gynaecology, dermatology, etc. pharmaceutical formulations containing water are favourable for safe application. Because Primycin dissolves in water at 20°C in 0.002 mass %, at 90 °C in 0.005 mass %, and even with 62.5 % ethanol only a 0.95 % solution can be prepared from it (Hungarian Patent Specification No.173.708), we performed systematic research to enhance the solubility of Primycin. To increase solubility auxiliary materials known from hydrotropy, such as urethane, acetamide, N-methyl-acetamide, N-dimethyl-acetamide, azophen, N-methyl-2-pyrrolidone, etc. are routinely used. /Münzel, K., Büchi J., Schultz O.E.: Galenisches Praktikum /Wiss.Verl. GmbH., Stuttgart, 1959/ p. 153 to 156/. Most of these materials can not be used e.g. in ophthalmol gy because of their irritative effects, and from many auxiliary materials it became known meanwhile that they are toxic, hence authorities prohibited their application, some others has not been permitted for usage.

We have learned in numerous experimental trials that surprisingly, cyclodextrins, cyclodextrin derivatives, r the mixtures thereof increase the solubility of Primycin to more than its tenfold and solubility can further be enhanced by isotonic 1.8 % boric acid solution usually applied in ophthalmology.

The subject of our invention are non-stoichiometric compounds with a 1:0.3 to 4.0 mole ratio of Primycin formed with a cyclodextrin, or a cyclodextrin-derivative, or mixtures thereof .

The non-stoichiometric compounds according to the invention can contain as cyclodextrin α-, and/or β-, and/or γ-, preferable β-cyclodextrin and/or as cyclodextrin-derivative maltosyl-β-cyclodextrin, dimethyl-β-cyclodextrin or randomly methylated β-cyclodextrin.

A further subject of the invention is a pharmaceutical composition containing as active ingredient 0.02 to 99.5 mass % of the non-stoichiometric compound with a 1:0.3 to 4.0 mole ratio of Primycin formed with a cyclodextrin, or a cyclodextrin-derivative, or with mixtures thereof, if desired 0.5 to 2.5 mass % of other active ingredients as anaesthetics, corticosteroids, antibiotics, chemotherapeutical active ingredients, if desired 1.2 to 1.8 mass % of boric acid, and in the quantity necessary to the 100 mass % other usually applied pharmaceutical auxiliary materials.

As for other active ingredient(s) the compositions according to the invention can contain tetracyclines as OTC, doxycycline, chloromycetin; aminoglycosid antibiotics as neomycin, sisomycin; furthermore sulphonamides, chloramphenicol, etc.

The compositions according to the invention are topic preparations formulated advantageously as aqueous solution, colloidal solution, gel, powder, aerosol, lotion for wound or plaster.

The compositions according to the invention contain, beside the active ingredient(s), when formulated as solution: as auxiliary materials preferably 1.2 to 1.8 mass % of boric acid, 0.1 to 2.0 mass % of a viscosity increasing substance, such as polyvinyl alcohol, hydroxypropyl-methyl cellulose and/or 0.01 to 0.03 mass % of chlorophyll and/or carotene; when formulated as gel they preferably contain 0.3 to 1.0 mass % of a gelation agent, preferably polyacrylate such as Carbopol, and related to 1 mass % of the gelation agent they contain as basic component 0.4 to 0.8 mass % of ammonia or ethylendiamine; when formulated as

powder they preferably contain as solid carrier lactose and/or dextran and/or aerosil, preferably a 99:1 ratio mixture of lactose and aerosil or a 1:0.1 to 10 ratio mixture of lactose and urea; when formulated as lotion for wound they contain 1 to 8 mass % of a pharmaceutically suitable essential oil such as cinnamon oil, cloves oil, eucalyptus oil, camphor, menthol and 90 to 98 mass % of oils usually applied in injection preparations.

The compositions contain the non-stoichiometric compound of Primycin formed with a cyclodextrin, or a cyclodextrin derivative or mixtures thereof preferable in micronised form. According to the invention the non-stoichiometric compounds with a 1:0.3 to 4.0 mole ratio of Primycin formed with a cyclodextrin, or a cyclodextrin-derivative, or mixtures thereof can be prepared by reacting Primycin with 0.3 to 4.0 mole cyclodextrin, or cyclodextrin-derivative, or with mixtures thereof

a.) in an aqueous and/or alcoholic medium, or

b.) in a 1.2 to 1.8 % aqueous boric acid solution, or

c.) in solid phase.

The eye drops according to our invention can preferably be produced by suspending 1 equivalent mass of finely powdered Primycin-sulphate and 1.5 to 2.5 mole of micronised beta-cyclodextrin in 1.4 to 1.8 % aqueous boric acid solution in such a ratio that the Primycin concentration of the solution should be 0.01 to 0.08 mass %, and reacting the solid components by keeping the suspension at boiling temperature under permanent stirring, and after cooling down the solution completing its volume with distilled water, and packaging it into appropriate containers used for pharmaceutical purposes.

According to an other preferred embodiment of the invention the mixture of 1 equivalent mass of Primycin and 0.3 to 4.0 mole ratio of beta-cyclodextrin is reacted with sufficient amount (preferably 7 to 20 fold volume) of 70 v/v % of ethanol calculated to the mass of the mixture, under continuous stirring, and boiling under reflux, the hot solution is then filtered, evaporated to dryness, and powdered. The dispersion of the Primycin-beta-cyclodextrin non-stoichiometric compound so obtained is suspended in 0.1 to 0.5 mass % ratio into hydrogel, which was adjusted to a pH value of 5 to 8 starting from a 0.8 to 1.2 mass % aqueous colloidal solution of a polyacrylic acid, preferable Carbopol and adding to it an inorganic or organic base, preferable ammonia, ethylendiamine, or lidocain base, then if desired according to the therapeutic purpose other active ingredient/s/, namely 0.5 to 2.0 mass % of corticosteroid, preferably hydrocortisone, 0.1 to 1.0 mass % antibiotic and/or chemoterapeutic

agents, 0.5 to 2.0 mass % local anaesthetis, 0.01 to 0.03 mass % chlorophyll and/or carotene are added and the hydrogel so obtained is used for therapeutic purposes.

In our examinations we completely dissolved 1 equivalent mass of Primycin-sulphate and respectively 0.3, 0.7, 1.0 , 1.5 , 2.5 and 3.0 mole of beta-cyclodextrin in sufficient amount of 70 v/v % ethanol at 60°C, and following evaporation of the solutions to dryness we examined the dispersions of the non-stoichiometric Primycin-beta-cyclodextrin compounds by X-ray diffraction method.

The powder diagrams were recorded on Philips pW 1840 type powder diffractometer, Cu-K_α -radiation, 30 KV and 30 mA excitation. The most characteristic lines of the powder diagrams are shown in the tables below:

Table 1.

X-ray diffraction powder diagram

1 mole Primycin + X mole Beta- cyclodextrin

Serial number	3.0 mole		2.5 mole		1.5 mole		1.0 mole		0.7 mole		0.3 mole	
	2θ	$\frac{I}{I_o}$	2θ	$\frac{I}{I_o}$	2θ	$\frac{I}{I_o}$	2θ	$\frac{I}{I_o}$	2θ	$\frac{I}{I_o}$	2θ	$\frac{I}{I_o}$
1	6.5	22	6.2	29	6.3	31	6.3	28	6.2	25	6.3	32
2	10.8	21	10.4	20	10.9	20	10.4	29	11	19	10.8	21
3	12.6	71	12.5	59	12.5	45	12.3	52	12.5	30	12.6	20
4	18	96	18	89	18	83	18.5	96	18.6	93	18.6	87
5	18.8	100	18.6	100	19.4	100	19.3	100	19.4	100	19.6	100
6	21.4	45	21.6	55	21.3	69	21.4	72	21.4	84	21.6	94
7	27	22	27.	16	27.3	17	26.5	20	26.8	21	26.7	13
8	35.2	26	34.6	24	35.3	17	34.8	19	34.7	13	34.8	15

Table 2.

X-ray diffraction powder diagram

1 mole Primycin + 2 mole Beta- cyclodextrin

Serial No.	2θ	$\frac{I}{I_o}$	Serial No.	2θ	$\frac{I}{I_o}$	Serial No.	2θ	$\frac{I}{I_o}$	Serial No.	2θ	$\frac{I}{I_o}$
1	2.9	7	9	14.5	22	17	21.2	73	25	29.2	12
2	4.5	23	10	15.1	34	18	22.4	59	26	30	11
3	6	21	11	15.9	26	19	22.7	64	27	30.8	22
4	8	27	12	17	71	20	24	32	28	31.6	18
5	8.5	12	13	17.4	96	21	24.8	36	29	32.4	11
6	10.4	41	14	18.6	100	22	25.3	40	30	34.7	37
7	11.3	26	15	19.4	98	23	26.8	46	31	36.5	18
8	13.3	94	16	20.7	84	24	28.3	16	32	37.5	14

Note: the hundredfold values of the $\frac{I}{I_0}$ relative intensities are displayed.

For comparative evaluation of the powder diagrams we present powder diagrams of beta-cyclodextrin /I/ and of Primycin /II/ in Figure 1 while the most important data of the powder diagrams in Table 3:

Table 3.

Serial Number	β - cyclodextrin		Primycin	
	2θ	$\frac{I}{I_o}$	2θ	$\frac{I}{I_o}$
1	4.2	71	2.7	29
2	12.2	100	6.3	27
3	19.2	46	11.1	23
4	20.4	80	19.6	97
5	23.9	44	20.8	99
6	25.3	90	21.5	100
7	26.8	57		
8	34.7	51		

From the test data it can be seen that the beta-cyclodextrin can be characterized with a well defined crystalline, while the Primycin with an X-ray-amorphous, non-crystalline solid structure. However, in the examined samples the peaks characteristic for the powder diagrams of the two components do not appear with an additivity corresponding to their mass ratio. The 2 θ° locations and intensity ratios of the peaks with 100 % intensity and those of the following very characteristic high intensity peaks have completely changed. In the examined dispersions the maximal intensity peaks can be found in the range of 18.6 to 19.6 2θ° indicating that the solid-structures of the dispersions are similar, practically they correspond to the isomorphic crystal forms.

Inclusion complex-binding ability of the beta-cyclodextrin is known /Szejtli J.: Cyclodextrins and their inclusion complexes (Publishing House of the Hungarian Academy of Sciences Budapest,1982)/. It was not known however, and we experienced in surprise, that by reacting the beta-cyclodextrin with Primycin in the stoichiometric ratio range of 0.3 to 0.4 it transforms into a dispersion with isomorphic solid state structure, characteristic for the non-stoichiometric compounds / Erdey-Grúz T., Fodorné Csányi P.: Rules of the Hungarian Chemical Nomenclature and Orthography (Publishing House of the Hungarian Academy of Sciences Budapest, 1972.Vol.I.p.92)/. According to rules of the UPAC th general formula

I. of the non-stoichiometric compound of Primycin / $C_{55}H_{102}N_3O_{17}$. 1/2 H_2SO_4 / and of beta-cyclodextrin /$C_{42}H_{70}O_{35}$/ is:

[{$C_{55}H_{102}N_3O_{17}$. 1/2 H_2SO_4}.{$C_{42}H_{70}O_{35}$}x] $(0.3<x<4.0)$.

Where X means 0.3 to 4.0.

The Setaram M-DSC.3 / Differential Scanning Calorimetry/ experiments are suitable for recognising incompatibilities due to interactions of the individual components (Figure 2.). In order of getting more accurate knowledge about the possible thermochemical changes accompanied with the thermal effect we made standard derivatogram experiments reported in the literature /G.Liptay Ed.: Atlas of Thermoanalytical Curves. Monograph./ Heyden and Son Ltd., London, New York, Rheine 1971/., using Q-Derivatograph type thermoanalytical instrument manufactured by MOM, weighing in about 100 mg substance, and applying a heating rate of $5°.min^{-1}$.

On the derivatograms of beta-cyclodextrin, of Primycin and of 1÷2 mass ratio Primycin + beta-cyclodextrin non-stoichiometric compound the enthalpy of the change accompanied with the first thermogravimetric loss of weight is endotherm on basis of the differential thermoanalytical /DTA/ curve. The mass ratio of this change corresponds to water loss of the samples. However in the case of the physical mixture on basis of the DTA and of the differential thermogravimetric curves a point characteristic for an unexpected thermochemical change could be recognised on this first curve at 69 /±1/ °C, which is in good accordance with the Setaram DSC experiment. This was followed by additional endotherm, exotherm and finally endotherm thermochemical changes on the derivatogram of Primycin + beta-cyclodextrin, which was not to find on the derivatograms of the starting Primycin and beta-cyclodextrin. So it can be stated that from the thermochemical point of view the behaviour of the non-stoichiometric compound according to the invention is different from what one would expect on the basis of the results obtained for the two starting components.

We examined the series of non-stoichiometric compounds by applying additional physicochemical methods. Because these compounds are formed in the solid phase / Erdey-Grúz T., Fodorné Csányi P.: Rules of the Hungarian Chemical Nomenclature and Orthography (Publishing House of the Hungarian Academy of Sciences Budapest, 1972.Vol.I.p.92)/. and the possible, mainly ligation chemical bonds between the components can be detected in solutions only with difficulty, we performed both infrared (IR) spectral tests, characteristic for chemical structure in the solid phase and nuclear magnetic resonance (NMR) tests, characteristic for the solution.

The IR spectra were recorded with a Bruker IFS28 type Fourier-transform instrument, using potassium bromide pellets technique. Of the series of the non-stoichiometric compounds we

want to focus on those three representative samples, where 1 mole quantity of Primycin /PR/ was reacted with 1, 2 and 3 mole quantities of beta-cyclodextrin /CD/, respectively. The samples were dissolved in boiling ethanol, then evaporated to dryness, and according to the 1, 2, and 3 mole ratios they were denoted as PR+CD-1, PR+CD-2 and PR+CD-3.

The table below shows wave numbers /cm^{-1}/ of the most characteristic peaks of the IR spectra:

PR	CD	PR+CD-1	PR+CD-2	PR+CD-3
843.2	947.3	846.2	846.8	846.8
923.6	1028.9	946.7	946.7	946.5
1087.4	1080.4	1029.4	1028.9	1028.9
1190.8	1157.4	1157.6	1157.6	1158.6
1461.3	1419.7	1457.2	1417.4	1420.1
1674.3	1653.8	1669.0	1667.7	1663.4
2932.1	2925.6	2929.2	2928.7	2928.7
3387.1	3388.3	3385.5	3389.5	3389.5

From the spectral data it can be seen, that the solid state chemical structures for the series of non stoichiometric compounds are practically identical, but wave numbers of characteristic peaks of the starting components and also the ratio of the transmittance values, not detailed here, slightly but characteristically differ. Non-stoichiometric compounds with mole ratios of 0.3 to 4.0 displayed similar results.

The NMR spectra of the same samples were recorded in deutero-dimethyl-sulfoxide solution on a Bruker AC - 400 type spectrophotometer.

In the NMR spectra of the tested samples protons of the starting CD exhibited multiplets in the chemical shift ranges of 3.2 to 3.9, 4.5 to 5.0 and 5.5 to 6.0 ppm, while most characteristic spectral peaks of the PR were multiplets in the range of 0.7 to 2.0 ppm. In the spectra of the samples PR+CD-1, -2, -3 in the same ranges the number of the characteristic multiplet peaks slightly differed from the rule of additivity. As a consequence of the ligation chemical bonds characteristic for the solutions of non-stoichiometric compounds, in our case

first of all due to hydrogen-bridge bonds, e.g. in the spectra of the PR and CD, in the range of 3.2 to 3.7 ppm the number of the multiplet peaks was altogether 17, whereas the same in the non-stoichiometric compound series was only 14. We also experienced a similar deviation in the case of non-stoichiometric compounds with mole ratios 0.3 to 4.0.

Next we examined the solubility of PR + CD. To 200 mg of Primycin so many mililiters of 1 % aqueous beta-cyclodextrin solution were added, that the PR + CD mole ratio should be 1+0.3, ...,1+4.0 and the samples were completed to 10 ml with water, then sealed into ampoules and kept at 100°C for 1 hour. After cooling the clear aqueous solution parts of the suspensions were examined by high pressure liquid chromatography /HPLC/ method. As reference the sample of the starting Primycin completely dissolved in ethanol /marked with "0" in the table/ was examined, too.

From the literature /J.Frank, et al: Tetrahedron Letters 28 2759 (1987)/ it is known that the Primycin produced by fermentation contains by nature analogous derivatives in different ratios marked with A, B, and C, as well as the -butyl, -pentyl, and -hexyl homologues thereof / marked in index with 1,2,3, /. The "0" mark column of the table below presents the natural ratio of the homologous compounds in the examined sample of Primycin expressed in per cents. For the series of non-stoichiometric compounds Table 4. presents the percentages of the most important homologues dissolved, related to the total amount of Primycin dissolved.

Table 4.

1 mole PR + CD mole	"0"	0.3	0.6	1.0	2.0	3.0	4.0
total dissolved PR μg/ml	-	253	233	275	352	248	269
A_1 Primycin %	72.3	78.5	80.2	80.4	80.4	79.3	79.1
A_2 Primycin %	8.6	9.3	8.98	8.4	1.4	1.2	1.1
A_3 Primycin %	2.0	0.1	0.1	0.3	2.4	2.5	2.5
B_1 Primycin %	4.7	2.6	2.6	2.4	7.8	8.6	9.0
$B_2+B_3+C_1+C_2+C_3$ %	12.2	9.5	8.2	8.5	8.0	8.4	8.3

From the data of the table turns out that surprisingly and not predictably, in the case of the non-stoichiometric compound the Primycin homologues are getting into the aqueous solution selectively and with significant differences.

Afterwards, solutions of the non-stoichiometric compounds with several ratios, and their combinations with boric acid were prepared and the microbiological activities of the solutions were compared with that of the Primycin-boric acid solution not containing beta-cyclodextrin. The following Table shows the compositions of the examined solutions and their apparent microbiological activities related to the unit mass:

Table 5.

10.0 ml aqueous solution contains			Primycin mg	increase of activity %
Primycin mg	cyclodextrin mg	boric acid mg	calculated on the basis of microbiological activity	
2.0	-	-	2.0	0
2.0	4.0	-	2.70	35.00
2.0	6.0	-	2.57	28.50
2.0	8.0	-	2.12	6.00
2.0	4.0	180	2.73	36.50
2.0	-	180	2.48	24.00
4.0	-	180	4.85	21.25
8.0	-	180	9.76	22.00
8.0	16.0	-	10.78	34.75

Microbiological activity has been determined in the usual way, by the so-called dilution method, applying Streptococcus faecalis ATCC 8043 test organism, and measuring spectrophotometric transmission at 570 nm, with a layer thickness of 4 cm.

The data if the table indicate that for the solutions containing 4.0, 6.0 and 8.0 mg cyclodextrins, which correspond to the non-stoichiometric compounds containing beta-cyclodextrin in 2, 3, and 4 mole ratio, the biological activity increased. Surprisingly and in a way which could not be calculated from the analogies in the literature /Nuppenau H.: Archiv. Pharm. Chemi. 67 1033 /1960/ either, the greatest apparent increase in activity was shown by the 2 mole ratio dispersion. 16 mg of beta-cyclodextrin increased the solubility of Primycin in a solution of 180 mg boric acid in 10 ml of water to forty-fold, while the apparent microbiological activity did not decrease.

According to our test experiences the non-stoichiometric compound of Primycin and 2 mole ratio of beta-cyclodextrin dissolved in combination with boric acid retained its favourable microbiological activity even if it contained 0.7 to 2.0 mass % polyvinyl alcohol or 0.2 to 0.8 mass % hydroxypropyl-methylcellulose as viscosity increasing substance.

According to a further preferred embodiment of our process a product formulated with alcohol-free hydrogel can be prepared in a way that 0.8 to 1.5 mass % of gelation agent, preferable polyacrylate, more preferable 1 mass % Carbopol 934 are homogenised with water at 20°C temperature, while stirring. Under constant stirring ammonium hydroxide solution in a quantity corresponding to 0.50 g NH_3 content -calculated with reference to 1 g of Carbopol -is dropped to the optically clear colloidal solution. This way a hydrogel with appropriate consistence is produced. In a similar way, dropping in place of ammonium hydroxide a solution containing 0.4 to 0.8 g of ethylenediamine -calculated with reference to 1 g of Carbopol - a hydrogel with appropriate consistence is produced too, reaching a pH value of 5 to 7.3. Into the gel so produced the micronised non-stoichiometric compound is homogenised containing 0.1 to 0.5 mass %, preferable 0.5 mass % Primycin and 1.7 to 2.3 mole ratio cyclodextrin.

We examined the biological potency of the hydrogel on deepithelized injured persons under camp hygiene conditions. For the purposes of comparison we treated one half of the deepithelized surface with 3 mass % clioquinol containing antiseptic ointment, and the other half with the above hydrogel containing 0.5 mass % Primycin, twice daily. By comparising the healing periods of the epithelial injuries without superficial infection with 6 similarly injured persons, we established that in the case of the hydrogel containing 0.5 mass % Primycin the healing required on the average 2.13 /variation coefficient ±0.74/ days, while in the case of the 3 mass % clioquinol containing ointment it required on the average 3.65 /

variation coefficient ± 1.08/ days. This means that of the two treatment modes, application of the Primycin hydrogel is by as much as 70 % more advantageous.

As for pharmaceutical products prepared according to our invention we combined the Wetol - lotion for wound composition / Issekutz B.: Pharmaceuticals and therapy (Rényi Károly Publishing House Budapest, 1944) vol.I. p. 387. and 388. Rp.906./ with the cyclodextrin non-stoichiometric compound containing 0.2 mass % Primycin. The examined lotion for wound contained 1.0 g from each of cinnamon-, cloves- and eucalyptus oil, 0.5 g camphor, 0.5 g menthol, 20 g cod liver oil, 20 g linseed oil, finally the non-stoichiometric compound prepared with 2 mole of beta-cyclodextrin containing 0.088 g of Primycin. As control a lotion for wound containing no Primycin was used.

We examined the lotion for wound on 21 different bacterium cultures /5 staphylococcus aureus, 5 streptococcus pyogenes, 5 enterococcus, 3 escherichia coli, 2 proteus mirabilis, 1 pseudomonas / in order to evaluate the antibacterial effect. From broth culture we prepared even bacterial lawn on blood-agar plate, onto which 15 minutes after inoculation 2 pcs. from each of the 2x2 cm MN 214 filter papers were placed soaked with Wetol-Primycin lotion for wound and with paraffin oil as control, respectively. Following 18 hours of incubation we determined on the basis of the number of colonies / 1 cm^2, that the Wetol lotion for wound containing no Primycin was ineffective against Enterococcuses, Gram-negative intestinal bacteria and Pseudomonases, exerted moderate effect against staphylococcus aureus and streptococcus pyogenes strains, the number of colonies was by 10 to 25 % lower than in case of the paraffin oil. On the other hand the Wetol lotion for wound combined with Primycin was proved to have antibacterial potency. In this case the number of the colonies was less than 3 %, within the margin of error of the method.

Further details of our invention are shown by the examples below, without limiting our claims to the examples.

EXAMPLES

Example 1.

Preparation of an eye drops containing 0.025 mass % of primycin

In a suitable, 50 l volume vessel 950 g boric acid were dissolved in 45 l water, in a portion of the solution 12.5 g of Primycin and 25.5 g of beta-cyclodextrin were reacted by boiling for 1 hour then it was poured into the 50 l vessel, completed to 50.00 l at 20°C, filtered and finally filled into eye drops containers.

Example 2.

Preparation of an ear drops containing 0.08 mass % of primycin

A solution was prepared by adding 20 l distilled water, 750 g boric acid, 40.8 g Primycin and 92.6 g beta-cyclodextrin successively into a 50 l suitable vessel and by heating it. After boiling the solution for 1 hour, it was cooled, diluted with water, then 0.80 kg polyvinyl alcohol were dissolved in it and its volume was completed to 50.00 l. After filtering the ready solution, it was filled into ear drops containers.

Example 3.

Preparation of nose - drops containing 0.05 mass % of primycin

5.0 g Primycin and 11.5 g beta-cyclodextrin were dissolved in 9 l 1.5 % aqueous polyvinyl alcohol solution while boiling, the solution was kept for 1 hour at boliling temperature then it was cooled. After completing the volume of the 20 °C solution to 10.00 l it was filled into nose-drops containers.

Example 4.

Preparation of hydrogel containing 0.5 mass % of primycin

500 g Primycin and 1110 g beta-cyclodextrin were reacted in sufficient amount of boiling 70 v/v % ethanol. The clear solution was evaporated, the dry residue was very finely powdered and it was homogenised with 100 kg hydrogel at the beginning in small portions. After adding 1.1 kg Carbopol to the hydrogel, it was transformed with 80 l distilled water to

colloidal solution, then under intensive stirring a solution of 470 g ethylenediamine in 10 l water was added to it, finally the gel was completed to 100 kg with distilled water.

Example 5.

Hydrogel, containing 0.5 mass % of primycin and 1 mass % of lidocain

It was worked as described in Example 4. with the difference that to the colloidal solution prepared with 1.1 kg Carbopol and 80 l water under intensive stirring the solution of 1 kg lidocain base in 2 kg ethanol was added and the gel was completed to 100 kg with distilled water.

Example 6.

Hydrogel, containing 0.5 mass % of primycin and 1.0 mass % of hydrocortisone

It was worked as described in Example 4. with the difference that 1.00 kg of very finely powdered hydrocortisone was homogenised to the Primycin-beta-cyclodextrin powder and this powder-mixture was worked into the 100 kg hydrogel.

Example 7.

Wound dusting powder containing 1.0 mass % of primycin

The very finely powdered dry residue of Primycin-beta-cyclodextrin prepared according to Example 4. was homogenised with 0.5 kg of Aerosil, then this powder mixture was completed with average finely powdered lactose to 50 kg and blended until reaching a uniform particle size. The powder mixture was filled into dusting powder- spreading containers.

Example 8.

Spray for treating wounds containing 0.5 mass % of primycin

It was worked as described in Example 4. with the difference that the very finely powdered Primycin-beta-cyclodextrin was homogenised with 25 kg hydrogel and the suspension gel was completed to 100 kg with distilled water. The viscous liquid was filled into containers fitted with atomizers.

Example 9.

Lotion for wound containing 0.5 mass % of primycin

Starting with small portions the very finely powdered dry residue of Primycin-beta-cyclodextrin prepared according to Example 4. was homogenised into the solution produced with the components of 1.000 kg from each of cinnamon-, cloves- and eucalyptus oil, 2.000 kg from each of camphor and menthol, 20.00 kg from each of cod liver oil and linseed oil, finally 53.00 kg oil for injections /Ph.Hg.VII./. The ready product was packed into containers fitted with droppers suitable for treating wounds.

Example 10.

Primycin wound plaster

80 g of Primycin and 220 g of gamma-cyclodextrin were dissolved in 3 l ethanol by boiling. After cooling the solution was soaked evenly with 10 kg of medicinal uncut mull and dried. The mull containing the active ingredient was cut to the desired size in 5 layers thickness. Packaging the measured units they were used to covering wounds as usual, or putting them on self-adhesive foil of appropriate dimension they were packed fitted with protective layer.

Example 11.

Combination spray containing 0.25 mass % of primycin

250 g Primycin, 250 g alpha- and 305 g beta-cyclodextrin were reacted in 5 l of 70 v/v % ethanol while boiling. After evaporating the solution the dry residue was homogenised in form of very fine powder in the following suspension: a colloidal solution was formed with 10 g Carbopol and 10 l water and 300 g of very finely powdered sulfamethylpyrimidine were suspended in it. The pH-value of the suspension was adjusted to 6.8 to 7.0 with ethylenediamine. The combination suspension containing Primycin was packed into bottles filled with hydrocarbon propellant and fitted with sprinkler known per se.

Example 12.

Primycin combination ear drops

It was worked as described in Example 2. with the difference that following the dissolution of polyvinyl alcohol and cooling of the solution 150 g neomycin sulphate having an activity of

at least 650 IU/mg were dissolved in it under steady stirring, then 500 g of very finely powdered hydrocortisone-acetate were suspended in the colloidal solution and afterwards the volume was completed to 50.00 l with distilled water. Following homogenisation, when necessary with the help of a colloid mill, it was packiged into ear drops containers.

Example 13.

Ointment containing 0.28 mass % of primycin

Using an equipment under pressure provided with stirrer, 280 g Primycin, 84 g alpha-, 260 g beta- and 100 g gamma-cyclodextrin were reacted in paste form with 150 ml water at 105°C for 1.5 hour by kneading. After cooling the dry product in the form of very fine powder was transformed to a suspension with 500 ml water and this suspension was homogenised with non-ionic emulgator ointment (Ph.Hg.VII.Vol.III. p.1881) completing to 100 kg. The ready product was filled into containers having the desired dimension.

Example 14.

Cream containing 0.28 mass % of primycin and lidocain

It was worked as described in Example 13. with the difference that the 500 ml aqueous suspension of Primycin and cyclodextrin was homogenised with a cream, previously prepared using a solution of 0.5 kg lidocain hydrochloride in 19.5 l of water and 79.5 kg of non-ionic emulgator ointment. The ready product was filled into containers having the desired dimension.

Example 15.

Paste containing 50 mass % of primycin

100 g Primycin and 66 g beta-cyclodextrin were dissolved in a sufficient volume of 70 v/v % ethanol by reacting them at the boiling temperature, then the solution was evaporated to dryness. The residue was formed with help of 25 g glycerol to a paste consistency product, having very fine particle size. The ready product was filled into containers for dental and oral surgery purposes.

Claims

1./

Non-stoichiometric compounds - with a 1:0.3 to 4.0 mole ratio - of Primycin or its components formed with a cyclodextrin or a cyclodextrin derivative or with mixtures thereof.

2./

The non-stoichiometric compound according to claim 1. containing as cyclodextrin α-, β-, or γ-cyclodextrin, as cyclodextrin-derivative maltosyl-β-cyclodextrin, dimethyl-β-cyclodextrin or randomly methylated β-cyclodextrin, preferable β-cyclodextrin.

3./

Pharmaceutical composition containing as active ingredient 0.02 to 99.5 mass % of non-stoichiometric compound - with a 1:0.3 to 4.0 mole ratio - of Primycin or its components formed with a cyclodextrin or cyclodextrin derivative or with mixtures thereof, if desired 0.5 to 2.5 mass % other active ingredients, as anaesthetics, antibiotics, chemotherapeutical active ingredients, if desired 1.2 to 1.8 mass % boric acid and in a quantity necessary to 100 mass % other usually applied pharmaceutical auxiliary materials.

4./

The pharmaceutical composition according to claim 3. containing 0.5 to 2.5 mass % of the non-stoichiometric compound of Primycin or its components formed with a cyclodextrin, or a cyclodextrin derivative, or with mixtures thereof.

5./

The pharmaceutical composition according to claims 3. in the form of a topic preparation, such as aqueous solution, colloidal solution, gel, ointment, powder, aerosol, lotion for wound or plaster.

6./

The pharmaceutical composition finished in solution form according to claims 3. to 5. containing as auxiliary material 1.2 to 1.8 mass % boric acid, 0.1 to 2.0 mass % viscosity increasing substance, as polyvinyl alcohol or hydroxypropyl-methyl cellulose, and/or 0.01 to 0.03 mass % cuticularization stimulating substance as chlorophyll and/or anti-oxidant as carotene.

7./

The pharmaceutical composition finished in gel form according to claims 3. to 5. containing 0.3 to 1.5 mass % gelation agent, preferable polyacrylate, as Carbopol, and if desired related to 1 mass % gelation agent, as basic component 0.4 to 0.8 mass % ammonia or ethylenediamine.

8./

The pharmaceutical composition finished in powder form according to claims 3. to 5. containing as solid carrier lactose and/or dextran, and/or polyvinyl-pyrrolidone, and/or urea, and/or aerosil, preferable a 99:1 ratio mixture of lactose and aerosil, or a 1: 0.1 to 10 ratio mixture of lactose and urea.

9./

The pharmaceutical composition finished in lotion for wound according to claims 3. to 5. containing as auxiliary material in 1 to 8 mass % therapeutically suitable essential oil, as cinnamon oil, cloves oil, eucalyptus oil, camphor, methanol and 90 to 98 mass % oil usual in injection preparations.

10./

The pharmaceutical composition according to claims 3. to 9. characterised by that they contain the non-stoichiometric compound of Primycin or its components formed with a cyclodextrin, or with a cyclodextrin derivative, or with mixtures thereof in micronised form.

11./

A process for preparing a non-stoichiometric compound with 0.3 to 4.0 mole ratio of Primycin or its components formed with a cyclodextrin, or a cyclodextrin derivative, or with mixtures thereof , characterised by reacting Primycin with 0.3 to 4.0 mole cyclodextrin or cyclodextrin-derivative or mixtures thereof

a.) in aqueous and/or alcoholic medium, or

b.) in 1.2 to 1.8 % aqueous boric acid solution, or

c.) in solid phase.

Figure 1

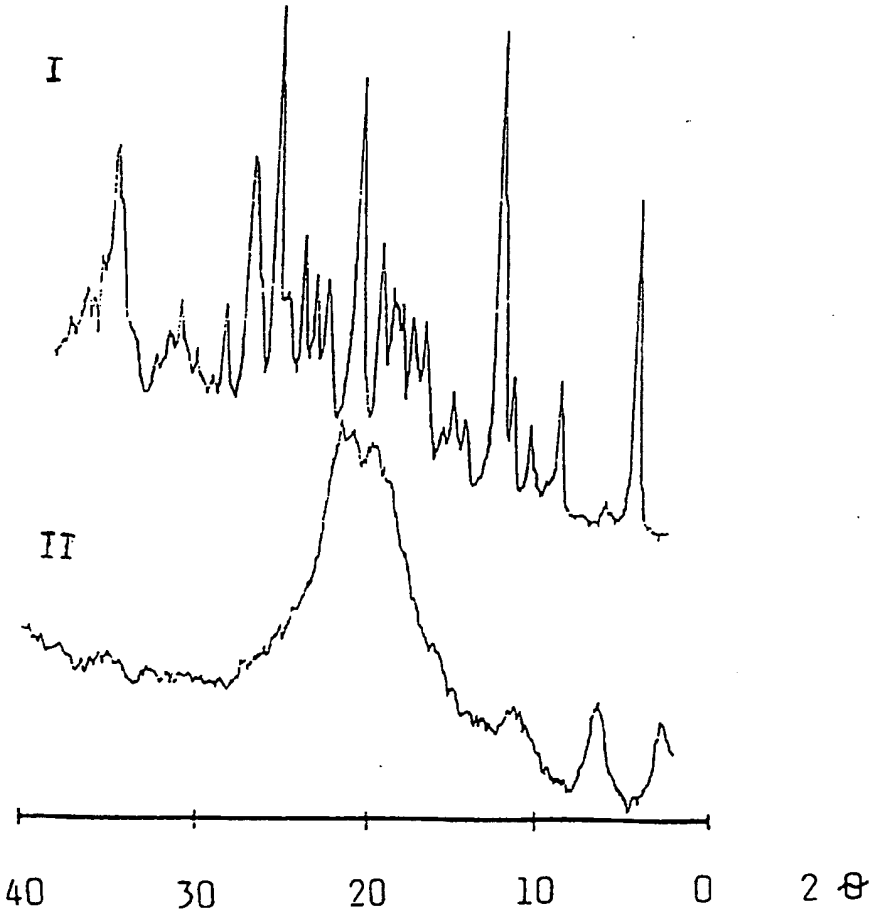
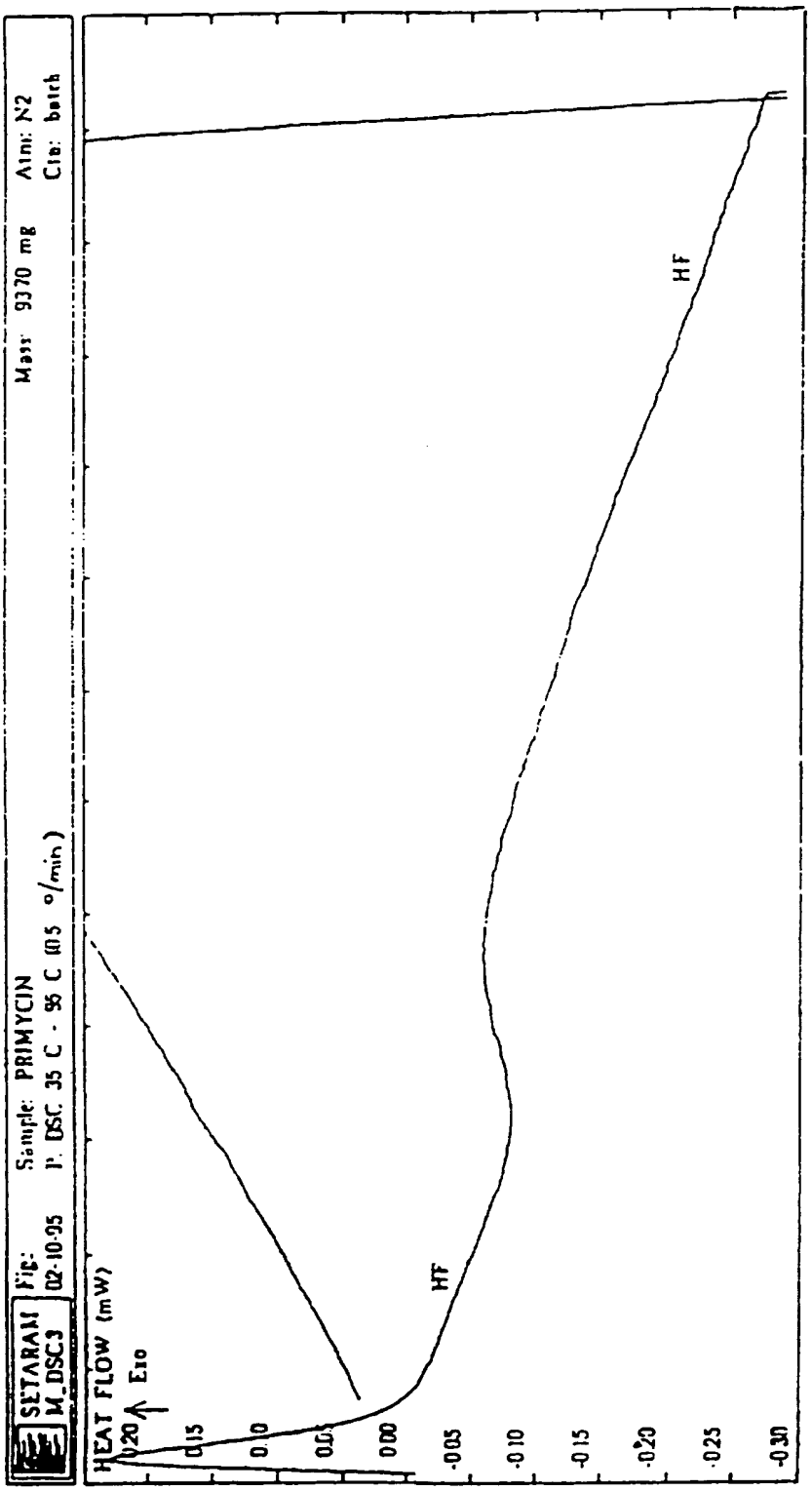


Figure 2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Mass: 9370 mg
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HEAT FLOW (mW)
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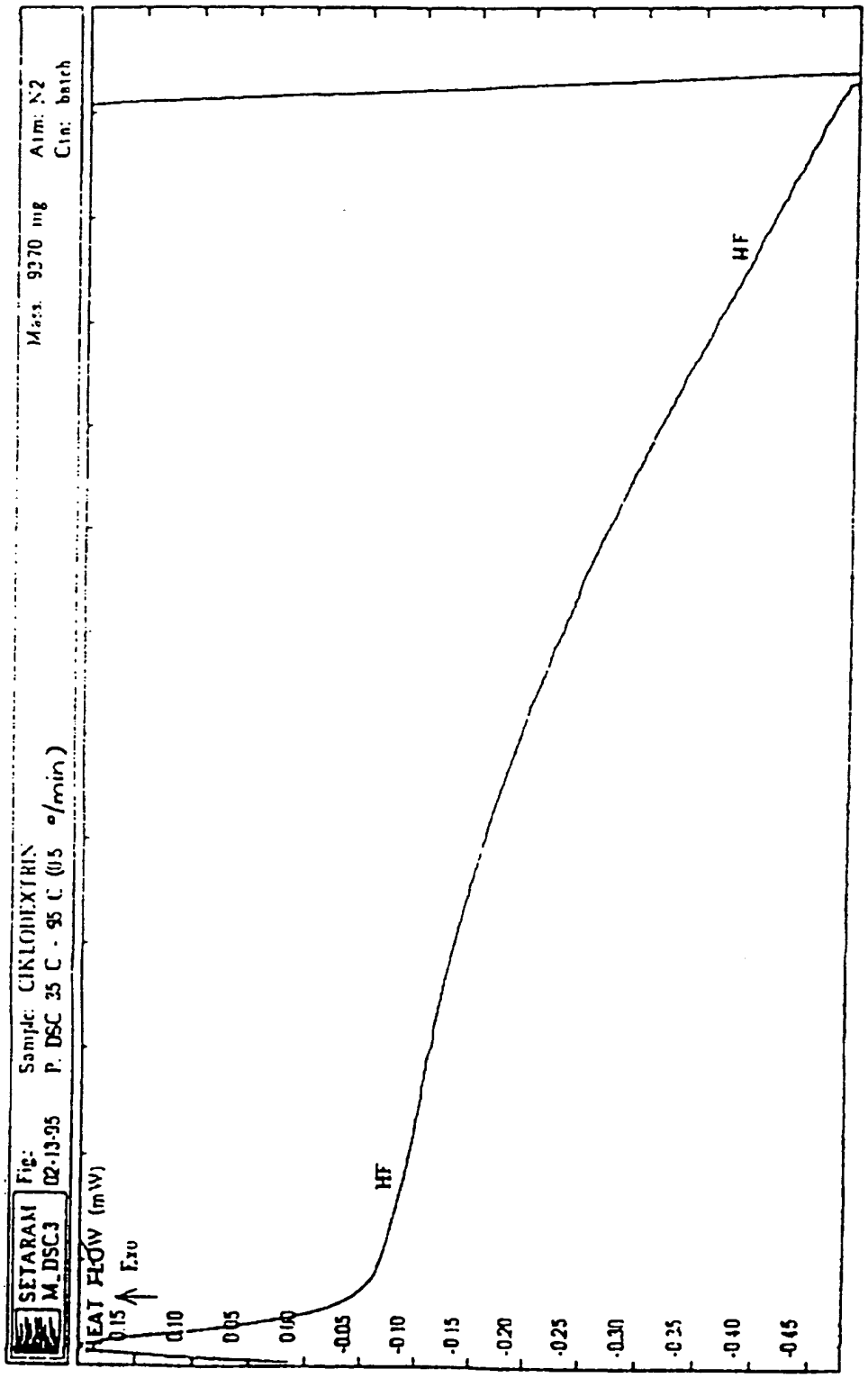
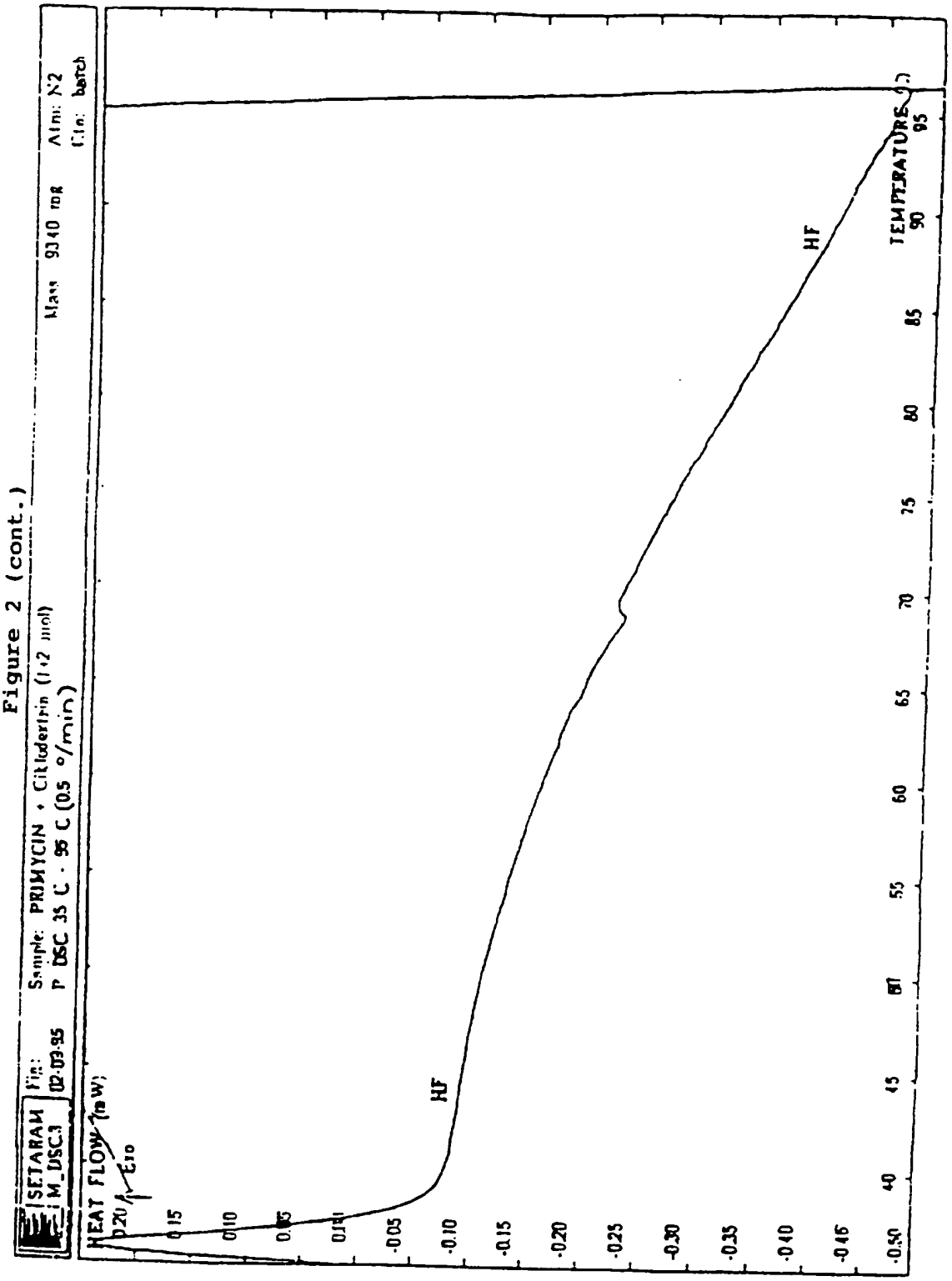


Figure 2 (cont.)

Figure 2 (cont.)



INTERNATIONAL SEARCH REPORT

Inte nal Application No
PCT/HU 97/00010

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C08B37/16 A61K47/40 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C08B A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 167 146 A (CHINOIN GYOGYSZER ES VEGYESZETI TERMEKEK GYARA RT) 8 January 1986 see page 9, line 20 - line 26 see page 15, line 19 - line 25; example 13 -----	1-5,11

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
10 July 1997	28. 07. 97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Mazet, J-F

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/HU 97/00010

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 167146 A	08-01-86	CA 1257254 A	11-07-89
		JP 61063695 A	01-04-86
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		US 4782141 A	01-11-88